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# Health Effects Assessment for Nitrobenzene

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# HEALTH EFFECTS ASSESSMENT FOR NITROBENZENE

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16. ABSTRACT

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with specific chemicals or compounds. The Office of Emergency and Remedial Response (Superfund) uses these documents in preparing cost-benefit analyses under Executive Order 12991 for decision-making under CERCLA. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary and reflect limited resources allocated to this project. The intent in these assessments is to suggest acceptable exposure levels whenever sufficient data are available. The interim values presented reflect the relative degree of hazard associated with exposure or risk to the chemical(s) addressed. Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, $RfD_S$ or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval. The RfD is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan. For compounds for which there is sufficient evidence of carcinogenicity, $q_1^*$s have been computed, if appropriate, based on oral and inhalation data if available.

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## PREFACE

This report summarizes and evaluates information relevant to a preliminary interim assessment of adverse health effects associated with nitrobenzene. All estimates of acceptable intakes and carcinogenic potency presented in this document should be considered as preliminary reflecting limited resources allocated to this project. Pertinent toxicologic and environmental data were located through on-line literature searches of the TOXLINE, CANCERLINE and the CHEMFATE/DATALOG data bases. The basic literature searched supporting this document is current up to May, 1985. Secondary sources of information have also been relied upon in the preparation of this report and represent large scale health assessment efforts that entail extensive peer and Agency review. The following Office of Health and Environmental Assessment (OHEA) sources have been extensively utilized:

U.S. EPA. 1980a. Ambient Water Quality Criteria for Nitrobenzene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Water Regulations and Standards, Washington, DC. EPA-440/5-80-061. NTIS PB81-117723.

U.S. EPA. 1983a. Reportable Quantity Document for Benzene, Nitro. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Emergency and Remedial Response, Washington, DC.

U.S. EPA. 1985. Health and Environmental Effects Profile for Nitrobenzene. Prepared by the Office of Health and Environmental Assessment, Environmental Criteria and Assessment Office, Cincinnati, OH for the Office of Solid Waste and Emergency Response, Washington, DC.

The intent in these assessments is to suggest acceptable exposure levels for noncarcinogens and risk cancer potency estimates for carcinogens whenever sufficient data were available. Values were not derived or larger uncertainty factors were employed when the variable data were limited in scope tending to generate conservative (i.e., protective) estimates. Nevertheless, the interim values presented reflect the relative degree of hazard or risk associated with exposure to the chemical(s) addressed.

Whenever possible, two categories of values have been estimated for systemic toxicants (toxicants for which cancer is not the endpoint of concern). The first, $RfD_S$ (formerly AIS) or subchronic reference dose, is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs during a limited time interval (i.e., for an interval that does not constitute a significant portion of the lifespan).

This type of exposure estimate has not been extensively used, or rigorously defined, as previous risk assessment efforts have been primarily directed towards exposures from toxicants in ambient air or water where lifetime exposure is assumed. Animal data used for $RFD_S$ estimates generally include exposures with durations of 30-90 days. Subchronic human data are rarely available. Reported exposures are usually from chronic occupational exposure situations or from reports of acute accidental exposure. These values are developed for both inhalation ($RfD_{SI}$) and oral ($RfD_{SO}$) exposures.

The RfD (formerly AIC) is similar in concept and addresses chronic exposure. It is an estimate of an exposure level that would not be expected to cause adverse effects when exposure occurs for a significant portion of the lifespan [see U.S. EPA (1980b) for a discussion of this concept]. The RfD is route-specific and estimates acceptable exposure for either oral ($RfD_O$) or inhalation ($RfD_I$) with the implicit assumption that exposure by other routes is insignificant.

Composite scores (CSs) for noncarcinogens have also been calculated where data permitted. These values are used for identifying reportable quantities and the methodology for their development is explained in U.S. EPA (1983).

For compounds for which there is sufficient evidence of carcinogenicity $RFD_S$ and RfD values are not derived. For a discussion of risk assessment methodology for carcinogens refer to U.S. EPA (1980b). Since cancer is a process that is not characterized by a threshold, <u>any</u> exposure contributes an increment of risk. For carcinogens, $q_1^*$s have been computed, if appropriate, based on oral and inhalation data if available.

## ABSTRACT

In order to place the risk assessment evaluation in proper context, refer to the preface of this document. The preface outlines limitations applicable to all documents of this series as well as the appropriate interpretation and use of the quantitative estimates presented.

The major toxicity study with nitrobenzene was a 90-day inhalation study using rats and mice (CIIT, 1984) in which hemolytic anemia and testicular lesions occurred at all levels (5, 16 or 50 ppm equivalent to 25, 81 or 252 mg/m³) in rats, and vacuolization of the adrenal cortex occurred at all levels (same as rats) in female mice. From the LOAEL of 25 mg/m³ in mice, an $RfD_{SI}$ and $RfD_I$ of 0.4 and 0.04 mg/day, respectively, were derived. A CS of 37.6 associated with testicular effects in rats was also calculated for 25 mg/m³.

An $RfD_{SO}$ and $RfD_O$ of 0.3 and 0.03 mg/day, respectively, were also derived from the LOAEL of 25 mg/m³ in mice, associated with adrenal changes.

## ACKNOWLEDGEMENTS

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## LIST OF ABBREVIATIONS

| | |
|---|---|
| ADI | Acceptable daily intake |
| CS | Composite score |
| DNA | Deoxyribonucleic acid |
| LOAEL | Lowest-observed-adverse-effect level |
| MED | Minimum effective dose |
| MTD | Maximum tolerated dose |
| NOAEL | No-observed-adverse-effect level |
| ppm | Parts per million |
| RfD | Reference dose |
| $RfD_I$ | Inhalation reference dose |
| $RfD_O$ | Oral reference dose |
| $RfD_S$ | Subchronic reference dose |
| $RfD_{SI}$ | Subchronic inhalation reference dose |
| $RfD_{SO}$ | Subchronic oral reference dose |
| $RV_d$ | Dose-rating value |
| $RV_e$ | Effect-rating value |
| TLV | Threshold limit value |
| TWA | Time-weighted average |

# 1. ENVIRONMENTAL CHEMISTRY AND FATE

The relevant physical and chemical properties and environmental fate of nitrobenzene are presented in Table 1-1. Synonyms for nitrobenzene are nitrobenzol, essence of mirbane and oil of mirbane.

Reaction with HO radical and photolysis appear to be the significant fate mechanisms in the ambient atmosphere. Based on an estimated reaction rate constant of $0.06 \times 10^{-12}$ $cm^3$/molecule-sec and an assumed atmospheric HO radical concentration of $10^6$ molecule/$cm^3$, the HO radical reaction half-life has been calculated to be ~133 days (U.S. EPA, 1985). In moderately polluted air, the half-life may decrease by a factor of 10 (U.S. EPA, 1985).

The aquatic half-life of nitrobenzene listed in Table 1-1 is based on an investigation by Zoeteman et al. (1980). In the aquatic environment, photolysis, volatilization and biodegradation are potentially significant fate processes. It is estimated that $\leq$3% of the nitrobenzene in rivers, lakes and ponds will remain in the sediments. Nitrobenzene should not bioaccumulate in aquatic organisms or ecologically magnify (U.S. EPA, 1985).

The soil half-life listed in Table 1-1 is the volatilization half-life of nitrobenzene obtained from a soil screening model. Potential exists for nitrobenzene contamination of groundwater, since this compound appears to be susceptible to significant leaching (U.S. EPA, 1985).

TABLE 1-1

Selected Physical and Chemical Properties
and Half-Lives for Nitrobenzene

| Property | Value | Reference |
|---|---|---|
| CAS number: | 98-95-3 | |
| Chemical class: | nitroaromatic hydrocarbon | |
| Chemical formula: | | |
| Molecular weight: | 123.06 | |
| Melting point: | 6°C | Windholz, 1983 |
| Boiling point: | 210-211°C | Windholz, 1983 |
| Vapor pressure:<br>at 20°C<br>at 25°C<br>at 30°C | <br>0.15 mm Hg<br>0.27 mm Hg<br>0.35 mm Hg | <br>U.S. EPA, 1985 |
| Water solubility:<br>at 20°C<br>at 25°C | <br>1900 mg/ℓ<br>2090 mg/ℓ | <br>U.S. EPA, 1985 |
| Log octanol/water partition coefficient: | 1.85 | Hansch and Leo, 1985 |
| Bioconcentration factor: | <10, golden orfe (*Leuciscus idus*); carp (*Cyprinus carpio*) 24, green algae (*Chlorella fusca*) 15, fathead minnow (*Pimephales promelas*) | U.S. EPA, 1985 |
| Soil/sediment adsorption coefficient: | 36-650 (estimated) | U.S. EPA, 1985 |
| Half-lives:<br>Air<br>Water<br>Soil | <br>NR<br>0.3-3 days (estimated)<br>NR | <br><br>U.S. EPA, 1985 |

NR = Not reported

## 2. ABSORPTION FACTORS IN HUMANS AND EXPERIMENTAL ANIMALS

### 2.1. ORAL

Rats that were treated with single 22.5 or 225 mg/kg doses of [$^{14}$C]-nitrobenzene by gavage eliminated ~61-66, 12-21 and 1-3% of the administered radioactivity in the urine, feces and expired air, respectively, in 72 hours (Rickert et al., 1983). Mice that were similarly treated with 225 mg/kg [$^{14}$C]-nitrobenzene eliminated a significantly lower percentage (~35%) of the administered radioactivity in the urine; similar amounts were eliminated in the feces and expired air. Bile collected from rats for 12 hours after administration of 225 mg/kg contained ~2-4% of the administered radioactivity; data after 72 hours were not reported, but it was indicated that biliary elimination of radioactivity was not fast enough to account for all of the fecal radioactivity found. This information indicates that gastrointestinal absorption of nitrobenzene is likely to be high ($\geq$80%), but that species differences in distribution or metabolism may exist as indicated by the mouse data.

### 2.2. INHALATION

Human pharmacokinetic data of Piotrowski (1967, 1977) and Salmowa et al. (1963) indicate that there is 80% retention of nitrobenzene during inhalation (U.S. EPA, 1980a; Beauchamp et al., 1982). Nitrobenzene, however, can be absorbed simultaneously through the skin; the ratio of dermal-to-inhalation absorption was reported to be 7:18 in an inductional setting (Piotrowski, 1967).

## 3. TOXICITY IN HUMANS AND EXPERIMENTAL ANIMALS

### 3.1. SUBCHRONIC

3.1.1. Oral. Pertinent data regarding the toxic effects of nitrobenzene from oral exposure could not be located in the available literature.

3.1.2. Inhalation. Groups of 10 male and 10 female Fischer 344 rats, Sprague-Dawley CD rats and B6C3F1 mice were exposed by inhalation to nitrobenzene at concentrations of 0, 5, 16 or 50 ppm (0, 25, 81 or 252 mg/m³) for 6 hours/day, 5 days/week for 90 days (CIIT, 1984). Increased methemoglobin levels were observed at ≥25 mg/m³ in Fischer rats, at ≥81 mg/m³ in CD rats and at 252 mg/m³ in the mice. Evidence of hematopoiesis and hemolytic anemia, including reticulocytosis, was observed at ≥81 mg/m³ in Fischer 344 rats and at 252 mg/m³ in the male CD rats, but not in the mice. Increased extramedullary hematopoiesis and hemosiderosis in the spleens were observed in both F344 and CD rats at ≥25 mg/m³, especially at the highest exposure level. Increased hemosiderosis also occurred in mice at ≥25 mg/m³. A significant increase in splenic weight was observed in rats exposed to ≥81 mg/m³ and in mice exposed to 252 mg/m³.

A "minimum or very slight degree" of toxic nephrosis was observed in ~50% of CD and Fischer 344 rats exposed to 25 mg/m³ (CIIT, 1984). Both the intensity and incidence of toxic nephrosis increased with increasing exposure levels in the rats. At 252 mg/m³, CD rats had increased kidney weights. Toxic nephrosis was not seen in the mice.

Hepatic lesions also occurred in the rats and mice. These included increased incidences of focal centrilobular necrosis at 252 mg/m³ in the F344 rats, hepatocellular hypertrophy and Kupffer cell pigmentation at ≥25 mg/m³ in the CD rats, periportal basophilia and enlarged nucleoli at ≥81

mg/m³ in the CD rats and centrilobular hepatocellular hyperplasia at ≥81 mg/m³ in the mice. The incidence and intensity of these effects generally increased with increased exposure levels.

Severe degeneration of the spermatogenic epithelium coupled with significantly decreased testicular weight and an absence of mature sperm in the epididymis was observed in 9/10 CD and 10/10 F344 rats that were exposed to 252 mg/m³ of nitrobenzene (CIIT, 1984). Severe spermatogenic epithelium degeneration with testicular atrophy occurred in 1/10 CD rats exposed to 25 mg/m³, but only very slight spermatogenic epithelial loss occurred in 2/10 males at 81 mg/m³. Testicular alterations were not observed in any of the mice.

Vacuolization of the zona reticularis was observed in the adrenal glands of all female B6C3F1 mice exposed to nitrobenzene at all levels (CIIT, 1984). Although the adrenal lesion increased in intensity with increased exposure levels, the clinical significance of adrenal gland vacuolization in the reticular zone is unknown.

3.2. CHRONIC

3.2.1. Oral. Pertinent data regarding the toxic effects of nitrobenzene from oral exposure could not be located in the available literature.

3.2.2. Inhalation. Pertinent data regarding the toxic effects of nitrobenzene from inhalation exposure could not be located in the available literature. However, a 2-year inhalation carcinogenicity study of nitrobenzene, based on the findings of the 90-day study summarized in Section 3.1.2., is currently being conducted by CIIT (Section 4.2.2.).

3.3. TERATOGENICITY AND OTHER REPRODUCTIVE EFFECTS

3.3.1. Oral. Fischer 344 rats that were given single oral doses of 300 or 400 mg/kg nitrobenzene in corn oil developed necrotic primary and

secondary spermatocytes with multinucleated giant cells in the seminiferous tubules after 1-5 days (Bond et al., 1981). Necrotic debris and decreased numbers of spermatozoa were observed in the epididymis within 3 days of treatment. Groups containing three animals were evaluated in this study, but these effects were not evident at lower doses (50-200 mg/kg).

3.3.2. Inhalation. Bio/Dynamics Inc. (1983) studied the fetotoxicity of nitrobenzene following inhalation exposure in New Zealand white rabbits. In this range finding study, pregnant rabbits were exposed to nitrobenzene at 0, 10, 40 or 80 ppm (0, 50, 200 or 400 mg/m$^3$) for 6 hours/day on days 7-19 of gestation. Methemoglobin levels were measured in five rabbits of each exposure group on gestation days 13 and 19 and in all rabbits on gestation day 20. On gestation day 20, the rabbits were sacrificed, a gross post-mortem examination was conducted, liver and kidney weights were recorded and the numbers of live fetuses, dead fetuses, resorptions and implantation sites were determined. Fetuses were not examined for malformations.

No adverse maternal effects on mortality, body weight, gross examination at necropsy, or kidney and liver weights were noted. Methemoglobin levels in rabbits exposed at 80 ppm were significantly higher than controls throughout the study. Methemoglobin levels in rabbits from the lower exposure group also tended to be elevated compared to controls, but the only significant difference was the 40 ppm exposed group on gestation day 20. No differences in the numbers of live fetuses, dead fetuses, resorptions and implantation sites were noted.

In the definitive developmental toxicity study with nitrobenzene in rabbits (Bio/Dynamics Inc., 1984), groups of 19-21 females were exposed to 0, 10, 40 or 100 ppm (0, 50, 200 or 500 mg/m$^3$) according to the same protocol described in the Bio/Dynamics Inc. (1983) study and were sacrificed

on gestation day 30. The only evidence of maternal toxicity was slight elevations in relative liver weight and 40-60% increases in blood methemoglobin concentrations at 10 and 100 ppm. A slight but not statistically significant increase in the incidence of embryonal absorptions was observed at 100 ppm. There were no effects on fetal body weight or crown-rump length and no treatment-related increase in the incidence of malformations or developmental variations.

Tyl (1984) exposed groups of 26 mated CD rats to nitrobenzene at 0, 1.0, 10.0 or 40.0 ppm (0, 5.0, 50.3 or 201 mg/m³) for 6 hours/day on days 6-15 of gestation; gestation day 0 was the day that a copulatory plug was found. Dams were sacrificed on gestation day 21. Parameters of maternal toxicity (clinical signs, body weight, gross necropsy, selected organ weights) and parameters of developmental toxicity (fetal body weights, and external, visceral and skeletal malformations) were examined. Maternal toxicity was manifested in the high dose group by reduced rate of body weight gain during the exposure period, but there were no differences in terminal body weights at sacrifice. Effects on body weight gain were not observed at 1.0 or 10.0 ppm. Rats at 10 and 40 ppm had a dose-related increase in absolute and relative spleen weights. Exposure to nitrobenzene had no effect on reproductive parameters or fetotoxicity. There was no significant increase at any exposure concentration in the number of litters containing one or more fetuses with malformations or variations. There was an increase, however, in the incidence of total malformations at 1.0, but not at 10 or 40 ppm. The investigator concluded that nitrobenzene was not teratogenic in this study.

Dodd and Kintigh (1985) investigated the effects of inhaled nitrobenzene in a 2-generation reproductive study in CD rats. Groups of 30 male and 30

female rats were exposed to 0, 1, 10 or 40 ppm (0, 5, 50 or 200 mg/m³), 6 hours/day, 5 days/week for 10 weeks before mating. Exposure continued 6 hours/day, 7 days/week through a 2-week mating period. Mated females (mating evidenced by a dropped vaginal plug) were exposed 6 hours/day through day 19 of gestation and allowed to deliver. Exposure of nursing dams resumed for 6 hours/day, 7 days/week starting on postpartum day 5 and continuing through postpartum day 20. $F_0$ males were necropsied at the end of the mating period. $F_1$ rats were selected and continued on the same exposure regimen as their parents. The $F_1$ groups were adjusted to 30 rats/sex. $F_2$ rats were sacrificed at weaning. The usual reproductive indices were monitored.

There were no effects on survival or lactation indices at any exposure level. Body weights of $F_1$ rats of both sexes at 40 ppm were slightly lower than controls at weaning. Fertility was significantly ($p<0.001$) decreased only at 40 ppm. The fertility indices in the $F_0$ generation were 53.3 and 10.0%, respectively, compared with 100.0% for $F_0$ and $F_1$ controls. The reduction in fertility was attributed to effects on the reproductive tracts of the males. At necropsy, $F_0$ males had reduced relative testicular and epididymal weights, testes that were grossly reduced in size and histologically observed seminiferous tubular atrophy and spermatocyte degeneration. These effects were noted only at 40 ppm; there were no treatment-related effects in females.

In order to investigate the reversibility of nitrobenzene-induced effects on the reproductive organs of the male, control and 40 ppm $F_1$ males were allowed a 9-week recovery period after which they were mated with unexposed virgin females. The fertility index after the recovery period had increased to 46.7%, which the investigator interpreted as evidence for the

reversibility of the nitrobenzene-induced lesions in the testes of exposed rats. At necropsy, $F_1$ males exposed to 40 ppm and allowed a 9-week recovery period had reductions in relative testicular and epididymal weights greater than those of the $F_0$ males. Lesions observed microscopically were similar to those observed in the $F_2$ males, but the degeneration of spermatocytes appeared to be less active.

### 3.4. TOXICANT INTERACTIONS

Human clinical and animal experimental evidence indicates that alcohol has a synergistic effect on nitrobenzene poisoning (Dorigan and Hushon, 1976; Rejsek, 1947; Smyth et al., 1969). Dorigan and Hushon (1976) cited a case in which ingestion of an alcoholic beverage triggered symptoms, including coma, of acute nitrobenzene poisoning in a worker who had apparently recovered from the effects of chronic nitrobenzene poisoning. Rejsek (1947) concluded that ingestion of one beer can precipitate an acute crisis in individuals suffering from subchronic nitrobenzene poisoning as long as 6 weeks after the disappearance of symptoms.

## 4. CARCINOGENICITY

### 4.1. HUMAN DATA

4.1.1. Oral. Pertinent data regarding the carcinogenic effects of oral exposure to nitrobenzene in humans could not be located in the available literature.

4.1.2. Inhalation. Pertinent data regarding the carcinogenic effects of inhalation exposure to nitrobenzene in humans could not be located in the available literature.

### 4.2. BIOASSAYS

4.2.1. Oral. Pertinent data regarding the carcinogenic effects of oral exposure to nitrobenzene in animals could not be located in the available literature.

4.2.2. Inhalation. Nitrobenzene was recommended for a carcinogen bioassay to the National Cancer Institute (Helmes et al., 1982), based on the suspicion that nitrobenzene is carcinogenic since it is the nitro analog of aniline and because of the substantial potential for human exposure. Nitrobenzene is not included, however, on the most current list of chemicals scheduled for testing by the National Toxicology Program (NTP, 1986).

CIIT is currently conducting a 2-year inhalation carcinogenicity study in which Fischer 344 and Sprague-Dawley rats are exposed to 0, 1, 5 and 25 ppm nitrobenzene (0, 5, 25 and 126 mg/m$^3$, respectively), and B6C3F1 mice are exposed to 0, 5, 25 or 50 ppm nitrobenzene (0, 25, 126 or 252 mg/m$^3$, respectively). This bioassay is based on the results of the 90-day inhalation study summarized in Section 3.1.2.

## 4.3. OTHER RELEVANT DATA

Nitrobenzene has been tested for mutagenicity in the Ames assay with Salmonella typhimurium strains TA92, TA94, TA97, TA98, TA100, TA1535, TA1537 and TA1538 with negative results (Garner and Nutman, 1977; Chiu et al., 1978; Shimizu et al., 1983; Ho et al., 1981; Haworth et al., 1983; Anderson and Styles, 1978; Miyata et al., 1981). These assays were conducted with and without added exogenous metabolic activation preparations by plate incorporation, spot test or, in one study (Hughes et al., 1984) by vapor exposure methods. Nitrobenzene was mutagenic in S. typhimurium TA98 but not TA 100 in the presence of metabolic activation and norharman, which is found in tobacco smoke (Suzuki et al., 1983).

Nitrobenzene did not cause an increase in unscheduled DNA synthesis in hepatocytes from gavage-treated rats (Mirsalis et al., 1982). It is reported in the abstract of a Russian study that nitrobenzene did not produce micronuclei or chromosome aberrations in bone marrow cells or dominant lethal mutations in mice following unspecified intragastric administration (Fel'dt, 1985).

## 4.4. WEIGHT OF EVIDENCE

The carcinogenicity of nitrobenzene has not been adequately tested in animals or sufficiently evaluated in humans. Pending the results of the current 2-year CIIT inhalation study, nitrobenzene should be classified as an IARC Group 3 and CAG Group D chemical, reflecting the inability to classify the chemical because of lack of data.

## 5. REGULATORY STANDARDS AND CRITERIA

ACGIH (1986) currently recommends a TWA-TLV of 5 mg/m³ (1 ppm) for an 8-hour nitrobenzene exposure, with a caution that cutaneous exposure can significantly contribute to total exposure. The TLV is based on occupational exposures that did not result in the development of headaches, methemoglobinemia or anemia in workers. OSHA (1985) proposed a TWA permissible exposure limit of 5 mg/m³ (1 ppm) for nitrobenzene.

U.S. EPA (1980a) recommended an ambient water quality criterion of 30 µg/L based on organoleptic effects. An ADI of 0.032 mg/day was calculated from a subchronic inhalation LOAEL of 25 mg/m³ (CIIT, 1984), which reflects hepatic, renal and testicular effects in rodents.

## 6. RISK ASSESSMENT

### 6.1. SUBCHRONIC REFERENCE DOSE ($RfD_S$)

6.1.1. Oral ($RfD_{SO}$). Subchronic oral toxicity studies of nitrobenzene could not be located in the available literature. An $RfD_{SO}$ can be calculated, however, from the subchronic inhalation LOAEL of 25 mg/m³ from the CIIT (1984) study (Section 6.1.2.).

Route-to-route extrapolation for nitrobenzene is appropriate because the extent of oral and inhalation absorption appears to be comparable (see Chapter 2) and because similar toxic effects (e.g., methemoglobinemia and liver and testis alterations) are produced by oral (Bond et al., 1981; Goldstein et al., 1984) and inhalation (CIIT, 1984) exposures. Using the same approach as in Section 6.1.2., but using an inhalation absorption factor of 0.8 (see Section 2.2.), the dose absorbed by mice exposed to 25 mg/m³ is estimated to be 4.64 mg/kg/day and is considered to be a LOAEL. Using an uncertainty factor of 1000, as in Section 6.1.2., the $RfD_{SO}$ is calculated to be 0.0046 mg/kg/day or 0.3 mg/day for a 70 kg human.

6.1.2. Inhalation ($RfD_{SI}$). An $RfD_{SI}$ can be derived from the CIIT (1984) study in which groups of 10 Fischer 344 and CD rats and B6C3F1 mice/sex were exposed to 0, 5, 16 or 50 ppm (0, 25, 81 or 252 mg/m³, respectively) nitrobenzene for 6 hours/day, 5 days/week for 90 days. The effects associated with the exposure are detailed in Section 3.1.2. In rats, hemolytic anemia and lesions of the spleen (extramedullary hematopoiesis), kidney (toxic nephrosis) and liver (hepatocellular hypertrophy and Kupffer cell pigmentation) occurred at 25 mg/m³. The incidences of these lesions were similar to controls and intensity was minimal at 25 mg/m³, but incidences and severity increased with increasing exposure levels. One of 10 male CD rats exposed to 25 mg/m³ had severe testicular epithelial

degeneration and atrophy; degeneration was minimal and without atrophy in 2/10 CD rats at 81 mg/m³ and severe with atrophy and lack of mature sperm in the epididymis in 9/10 CD rats and 10/10 Fischer 344 rats at 252 mg/m³. Increased methemoglobin levels were also observed at ≥25 mg/m³ in Fischer rats. In mice, increased hemosiderosis in the spleen and vacuolization of the zona reticularis of the adrenal gland in females occurred at ≥25 mg/m³; the incidence and intensity of these effects increased with higher exposure concentrations. No liver, kidney or testicular effects were observed in the mice at 25 mg/m³.

The 25 mg/m³ exposure level is judged to be the LOAEL in rats because of the hemolytic anemia and spleen, kidney, liver and testis alterations. The testicular alterations at 25 mg/m³ (and 81 mg/m³) are treatment-related in a particularly sensitive animal in the more sensitive rat strain (CD). Although the Fischer rats also had increased serum methemoglobin levels and evidence of hemolytic anemia at 25 mg/m³, this effect in the rat may be an inappropriate basis for deriving an $RfD_{SI}$, since the TLV of 5 mg/m³ (ACGIH, 1986) is designed to be protective against methemoglobinemia and hematological effects in humans. This exposure level (25 mg/m³) is also considered to be the LOAEL in mice because of the spleen and adrenal cortex alterations; however, the toxicological significance of the adrenal effect (increased vacuolization of the cortex) is unknown.

The daily doses to which the animals were exposed are estimated by multiplying the exposure level by 6 hours/24 hours and 5 days/7 days to adjust to continuous exposure and by the inhalation rates (assumed to be 0.223 m³/day for rats and 0.039 m³/day for mice) (U.S. EPA, 1980b) and by dividing by the reference body weights (assumed to be 0.35 kg for rats and 0.03 kg for mice). These calculations show that at the LOAEL (25

mg/m³), the rats and mice were exposed to 2.84 and 5.80 mg/kg/day, respectively. Using the higher LOAEL of 5.80 mg/kg/day and an uncertainty factor of 1000 (10 for interspecies extrapolation, 10 to estimate a NOAEL from a LOAEL and 10 to protect the most sensitive humans), the $RfD_{SI}$ is calculated to be 0.0058 mg/kg/day or 0.4 mg/day for a 70 kg human.

6.2. REFERENCE DOSE (RfD)

6.2.1. Oral ($RfD_O$). Chronic oral toxicity data for nitrobenzene could not be located in the available literature. An $RfD_O$ for nitrobenzene can be calculated, however, from the subchronic inhalation LOAEL of 25 mg/m³ (CIIT, 1984) by the approach used to calculate the $RfD_{SO}$. By using the absorbed dose for mice estimated in Section 6.1.1. (4.64 mg/kg/day) with an additional uncertainty factor of 10 to approximate chronic exposure (total uncertainty factor of 10,000), the $RfD_O$ is calculated to be 0.0005 mg/kg/day or 0.03 mg/day for a 70 kg man (U.S. EPA, 1986).

6.2.2. Inhalation ($RfD_I$). Chronic inhalation toxicity data for nitrobenzene could not be located in the available literature. Pending the outcome of the current CIIT chronic toxicity study, an $RfD_I$ for nitrobenzene can be calculated from the subchronic inhalation LOAEL of 25 mg/m³ (CIIT, 1984) by using an approach identical to that in Section 6.2.1. With an additional uncertainty factor of 10 to approximate chronic exposure, the $RfD_I$ is calculated to be 0.0006 mg/kg/day or 0.04 mg/kg/day for a 70 kg human.

CSs for nitrobenzene can be calculated from the LOAEL (25 mg/m³) associated with the hemolytic, liver, kidney and testicular effects (252 mg/m³) identified in the CIIT (1984) subchronic inhalation rat study (Section 6.1.2.). Equivalent animal doses were estimated from the exposure concentration by multiplying by 6 hours/24 hours and 5 days/7 days to adjust

to continuous exposure, by the rat inhalation rate of 0.223 $m^3$/day (U.S. EPA, 1980b) and by dividing by the reference body weight of the rats (0.35 kg). The dose was then divided by an uncertainty factor of 10 to approximate chronic exposure. MEDs were calculated by multiplying the equivalent animal dose by the cube root of the ratio of reference animal body weight to human body weight (70 kg) and by the human body weight to express the MED in units of mg/day.

Since the MED is the same for all the effects in rats in this study, differences in CSs would reflect differences in severities of the effects (i.e., in the $RV_e$ assigned). The testicular alterations are the most serious effects and warrant an $RV_e$ of 8, associated with decreased reproductive capacity; although absence of mature sperm in the epididymis was reported only at 252 mg/$m^3$, this manifestation is consistent with the effects reported at 25 mg/$m^3$. A CS of 37.6 is based on the testicular effects in rats.

The CS calculated for the testicular effects (37.6) differs slightly from that (25.6) calculated by U.S. EPA (1985). The U.S. EPA (1985) derivation calculated the MED from the 81 mg/$m^3$ exposure level, which is not the LOAEL identified in the risk assessment section of the same report. The LOAEL for testicular effects was determined to be 252 mg/$m^3$ by U.S. EPA (1985), but additional consideration indicates that the testicular effects at 25 mg/$m^3$ are not anomalous, as indicated in Section 6.1.2.

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## APPENDIX

### Summary Table for Nitrobenzene[a]

| | Species | Experimental Exposure/Dose (mg/kg/day) | Effect | Reference Dose (RfD or $RfD_S$) |
|---|---|---|---|---|
| Inhalation | | | | |
| $RfD_{SI}$ (formerly AIS) | mouse | 5 ppm (25 mg/m³) 6 hours/day, 5 days/week for 90 days (5.8 mg/kg/day) | Vacuolization of adrenal cortex | 0.4 |
| $RfD_I$ (formerly AIC) | mouse | 5 ppm (25 mg/m³) 6 hours/day, 5 days/week for 90 days (5.8 mg/kg/day) | Vacuolization of adrenal cortex | 0.04[b] |
| Maximum CS | rat | 5 ppm (25 mg/m³) 6 hours/day, 5 days/week for 90 days (5.8 mg/kg/day) ($RV_d$ = 4.7) | Testicular atrophy and degeneration of spermatogenic epithelium ($RV_e$ = 8) | 37.6 |
| Oral | | | | |
| $RfD_{SO}$ | mouse | 5 ppm (25 mg/m³) 6 hours/day, 5 days/week for 90 days (5.8 mg/kg/day) | Vacuolization of adrenal cortex | 0.3 |
| $RfD_O$ | mouse | 5 ppm (25 mg/m³) 6 hours/day, 5 days/week for 90 days (5.8 mg/kg/day) | Vacuolization of adrenal cortex | 0.03[b] |

[a]Source: CIIT, 1984

[b]An additional uncertainty factor of 10 was applied to the $RfD_S$ to approximate chronic exposure.